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Application of Cysteine, Methionine and Amino Acid Containing Fertilizers to Replace Urea: The Effects on Yield and Quality of *Broccoli*

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Abstract

A greenhouse experiment was carried out to determine the influence of replacing 80% of urea in broccoli fertilization with L-cysteine and L-methionine foliar spray at two concentrations 100 and 200 mgL-1 and amino acid containing fertilizer (AA) at 750 and 1500 mgL-1 on yield, nitrate accumulation, dry weight of head, vitamin C, phenolic compounds and total acidity. Replacing 80% of urea with AA increased the total acidity significantly ($P \leq 0.05$). Elimination of 80% urea but foliar spray of cysteine (100 and 200 mgL-1), methionine (200) the percentage of dry weight significantly ($P \leq 0.05$). Urea (250 kg ha-1) plus amino acids and amino acid Alone in Comparison with control (just 50 kg ha-1 urea) increased significantly ($P \leq 0.05$) Yield. The content of vitamin C, decrease significantly ($P \leq 0.05$) by the urea (250 kg ha-1) application with amino acids. Application of amino acid containing fertilizer (750 and 1500 mgL-1) along with urea (250 kg ha-1) increased nitrate accumulation.

Keywords: Urea; Aminoacid; Cysteine; Methionine; *Broccoli*

Introduction

Broccoli (Brassica oleracea var. italica) is a member of Brassicaceae family having many important health beneficial vegetables such as cauliflower, cabbage, brussels sprouts and kale. Cancer research center of USA indicated that *broccoli* has several anti-cancer effects. It is an ideal diet food because of its nutritional content. A 100 g *broccoli* has 40 calories, 5 g protein, 1 g fat,10 mg 0.14 mg B1, 0.3 mg B2, 1.2 mg Niacin, 50 mg calcium carbohydrate, 3880 IU vitamin A, and 1.7 mg iron One of the most appealing characteristics of *broccoli* is its high vitamin C content which has been reported to 82-140 mg [1]. Plant Growth regulating substances (PGRs) were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth. PGRs also play important roles in plant adaptation to stressful environments. Their common role is to serve as building blocks of proteins, which exert manifold functions in plant metabolism, and as metabolites and precursors they are involved in plant defense, vitamin, nucleotide and hormone biosynthesis, and as precursors of a huge variety of secondary compounds. One way or the other, as active catalysts or as precursors, amino acids are essentially involved in all metabolic, regulatory, and physiological aspects of plant metabolism. Amino acid can serve as the sole source of nitrogen, which can be taken more rapidly than inorganic nitrogen. While, exogenous amino acids decreased both ammonium influx and transporter transcript in root tissue. Sulfur is a macronutrient that is essential for plant growth and development. The most abundant form of sulfur in nature and the source of sulfur for plants is sulfate; this form is reduced and assimilated into Cysteine. In addition to its role as an amino acid in proteins, Cysteine functions as a precursor for a huge number of essential biomolecules, such as vitamins and cofactors [2].

Antioxidants like glutathione, which is regarded as the major determinant of cellular redox homeostasis and many defense compounds [3]. All of these biomolecules contain sulfur moieties that act as functional groups and are derived from Cysteine In plants, cysteine biosynthesis plays a central role in fixing inorganic sulfur from the environmental provides the only metabolic sulfide donor for the generation of methionine, glutathione, phytochelatins, iron-sulfur clusters, vitamin cofactors, and multiple secondary metabolites. O-Acetylserine sulfhydrylase (OASS) catalyzes the final step of cysteine biosynthesis, the pyridoxal 5-phosphate(PLP)-dependent conversion of O-acetylserine into cysteine. Cysteine proteinases are potentially responsible for both low temperature and drought tolerance [4]. Cysteine is the metabolic precursor of essential biomolecules such as vitamins, cofactors, antioxidants and many defense compounds. The last step of cysteine metabolism is catalyzed by O-acetylserine (thiol) lyase (OASTL), which incorporates reduced sulfur into O-acetylserine to produce cysteine. In Arabidopsis thaliana, the main OASTL isoform OAS-A1 and the cytosolic desulfhydrase DES1, which degrades cysteine, contribute to the cytosolic cysteine homeostasis. In addition to its structural role in proteins, cysteine functions as a precursor for essential biomolecules, such as vitamins and cofactors antioxidants, such as glutathione and many defense compounds, such as glucosinolates, thionins or phytoalexins. Cysteine is synthesized in plants in the cytosol, plastids and mitochondria by the sequential action of the enzymes serine acetyltransferase which synthesizes the intermediary product O-acetylserine (OAS), and O-acetylserine(thiol)lyase which combines a sulfide molecule with an OAS molecule to produce cysteine [5].

Maxwell and Kieber indicated the link of methionine to the biosynthesis of growth regulating substances, e.g., cytokinins, auxins and brassinosteroids in plants [6]. *Broccoli* (*Brassica oleracea* var. *italica*) crop requires high supply of N for optimum yield [7]. It is known that fertilization is critical for a successful *broccoli* production. The aim of the present study was to investigate the effect of different sources of amino acid on growth, yield and some physical and chemical properties of *broccoli* plant. Amino acids can be directly absorbed by the roots and positive interaction between amino acids and micronutrients in the rhizosphere of nature has been observed that the absorption is better. Spraying with amino acid led to increase the absorption and decrease leaching of nitrogen from the soil. The use of amino acids alanine, serine,

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tyrosine, phenylalanine and hydroponic culture of tomatoes increases the concentration of calcium, potassium, magnesium, iron, copper and manganese in the plant. Root by removing amino acids facilitates the absorption of nutrients from the roots to them. This corresponds to a change in the amino acid to enter the cell membrane elements [8].

The objective of this research was to determine the effects of partial replacement of urea with different amino acids on *broccoli* growth, nitrate accumulation, yield and some chemical properties of *broccoli*.

Materials and Methods

The experiments were conducted on Shiraz University. The cultivar Green Parasol supplied by Takii Seed Company, Kyoto, Japan was used as experimental material. The cultivar is early maturing (72-75 day after transplanting) having uniform, dark green head. L-Cysteine and L- methionine supplied by Merck Company. Amino acid fertilizer supplied by Bazargankala (Iran). Spraying treatments were started after four weeks from planting date and every 15 days for 3 times through the growth season.

At harvest time (72 days old) the total weights of head resulting from each experiments plot were recorded and the yield calculated.

Chemical analysis

Phenolic compounds were determined according to the methods described by Ismail [9]. Nitrates in the tissue determined according to the methods described by Tabatabaee [10]. Acidity and vitamin C content were determined according to Horwitz [11] and Ting [12] respectively.

The experimental trails were conducted in soil using drip irrigation system. Chemical analysis and physical properties of the experimental soil are shown in Table 1.

Statistical analysis

The experiments were conducted in randomized complete block design with three repelications. The data were analyzed through analysis of variance technique using SAS 9.1.3 portable by SAS institu procedures. The significant variables were subjected to mean separation using LSD test at 1% significance level.

Results and Discussion

Phenolic compounds

Where nitrogen was not applied, all amino acid treatments at 100 mg.L managed to increase the phenolic content and resulted in significant differences with the control (P<0.01). Where nitrogen was applied, it was observed that the methionine treatment at 100 mg.L acted to reduce the phenolic content and this was significant in comparison to the control (P<0.01). It was further observed that those plants which neither received nitrogen nor amino acids had the lowest amount of phenolic contents (Table 1). It is known that phenolic compounds are derived from amino acids [13]. It has been reported that putrescine and glutathione had incremental effects on the phenolic content in onions [14].

Phenylalanine, ornithine and proline are claimed to have increased phenolic substances in the yarrow [15]. Considering the fact that amino acids are involved in the production of phenolic compounds and while knowing that amino acids like tyrosine, tryptophan and proline are actually incorporated into the structure of phenols, it is reasonably anticipated that the application of amino acids on the plant can promote the synthesis of phenolic substances. Comparison of the effects of treatment and nitrogen compounds as well as their interactions to rate.

Nitrates in the tissue

Results (Table 2) show that, where nitrogen was not added, the application of cysteine at 200 mg.L resulted in a significant difference compared to the control, regarding nitrate content (P<0.01). Where nitrogen was added, the amino acid fertilizer at 750 mg.L led to a marked difference with the control; by considering the table, it can be understood that cysteine at 200 mg.L and methionine at both its concentrations acted to reduce the nitrate content in comparison to the control. It is common knowledge that the application of amino acids on plants can improve their ability in absorbing and transporting mineral elements [16]. Therefore the concurrent application of amino acids and nitrogen can further enhance nitrogen uptake and lead to the accumulation of nitrates within plant tissues [17].

However, the sole application of amino acids acts to reduce the level of nitrates in the tissues. Based on the table pertaining to the reduction in nitrate levels, with regard to methionine, the decline in nitrate levels can be attributed to the positive effect of methionine on nitrate reductase. Methionine encourages the activity of that enzyme and thus reduces the level of nitrate in the tissues [18]. By entering the citric acid cycle, amino acids can boost the production of NADH which is necessary for nitrate reductase to remain active and can increase the enzyme's activity [10]. As the activity of nitrate reductase is heightened, the level of nitrates decline in the tissues. There are several reports regarding the direct blockade of nitrate transportation via the help of amino acids [19].

Acidity

When nitrogen was not added, methionine application at 100 mg.L resulted in the highest acid and had a substantial difference compared to the control group (P<0.01). The addition of nitrogen, however, caused all treatments to increase the acidity of tissues and this was also significantly different from the control group (P<0.01). Since amino

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	28.464 b-e	26.791 d-f
Cysteine 200	35.254 a	23.332 ef
Methionine 100	32.788 ab	30.980 a-d
Methionine 200	28.107 b-e	26.395 d-f
AA 750	31.749 a-c	24.008 ef
AA 1500	27.647 c-e	24.962 ef
control	21.692 f	14.335 g

AA (aminoacid containing fertilizer), Treatments that are shared with other letters are significant (P<0.01).

Table 1: Effect of source and amino acid concentration on Phenolic compounds (μg.ml).

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	39.8 c-f	44.7 b-d
Cysteine 200	34.3 e-g	41.2 c-e
Methionine 100	29.7 g	46.6 bc
Methionine 200	27.7 g	39.5 c-f
Aa 750	42.1 c-e	53.3 ab
Aa 1500	43.7 b-e	57.0 a
Control	31.4 fg	35.3 d-g

AA (aminoacid containing fertilizer). Treatments that are shared with other letters are significant (P<0.01).

Table 2: Effect of source and amino acid concentration in Nitrates (g/kg).

acids can enter the citric acid cycle [14], it is highly probable that the influence on acid content happens through the citric acid cycle, thereby altering the acidity level of tissues. Furthermore, we observed that the level of ascorbic acid increased as another result of applying amino acids.

Vitamin C

According to the results on Tables 3 and 4, in the absence of nitrogen application, cysteine at 100 mg.L led to the highest level of vitamin C in the tissues. However, the amino acid fertilizer at both its concentrations made for the least vitamin C level. Both results were significantly different from the control group (P<0.01). In the presence of nitrogen application, however, it was observed that all of the separate amino acid treatments, except for one, led to reductions in the vitamin C content and this was significantly different from the control group (P<0.01). The exception was methionine at 100 mg.L. Based on the synthetic pathway of ascorbic acid and the fact that the synthesis of ascorbic acid necessitates sugars, it can be postulated that whatever factor which increases the sugar (or glucose) content in plant tissues can, in turn, increase the vitamin C content [20].

And yet the differences observed in the level of vitamin C in this study can be related to variations in genotype, climatic fluctuations and the state of plant nutrition. It has been reported that nitrogen fertilizers do not influence the vitamin C content in *broccoli*. However in cauliflower, when the amount of nitrogen fertilizers are increased from 80 kilograms to 120 kilograms per ha, the vitamin C content decreases by 7% [21].

Yield

According to the results on Table 5 except for cysteine at 200 mg l. all of the separate amino acid treatments had incremental effects on yield when there was no application of nitrogen fertilizer, and this was significant compared to the control group (P<0.01). A study on garlic revealed that the combination of four amino acids, Cysteine, Alanine, Glycine and Arginine could improve yield. Some amino acids like tryptophan are recognized to be the precursors of phytohormones. Amino acids are also involved in the synthesis of amines, alkaloids, terpenoids, enzymes and vitamins. They are essential to the maintenance of cellular growth and are a type of buffer substance while also serving as sources of carbon and energy. Fertilizers that contain amino acids enhance the plant's ability to absorb water and minerals from the soil and thus provide for a better yield. The foliar application of amino acids on potatoes, summer squash and garlic has resulted in more vigorous growth and higher yields. Arginine enhanced the yield of onions [16]. Foliar applications of putrescine and glutathione on onion plants have resulted in the same outcome [14]. While taking into account the aforementioned argumentations, it can be concluded that amino acids increase the plant's chlorophyll content and contribute to the saving of energy, thereby bolstering up the plant's productivity. Therefore, when the yield that is obtained through the application of nitrogen fertilizers nearly equates to that obtained through amino acids, the latter can be a suitable substitute for nitrogen.

Percentage of dry weight

Results from the Table 6, show that, with regard to dry weight, there is a significant difference between the joint application of nitrogen fertilizer plus amino acid fertilizer and the sole application of amino acids (P<0.05). In other words, the use of nitrogen fertilizer has reduced the percentage of dry weight because of its role in rearing swollen, water-filled tissues due to excessive vegetative growth. When urea and nitrates are applied as fertilizers, a portion of the plant's carbon structure is spent on the molecular reduction of these fertilizers, which can lead to decreases in the dry weight [10]. In this study, the heaviest dry weight was achieved in the control group which received neither amino acids nor nitrogen.

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	0.45 e	0.43 e
Cysteine 200	0.52 bc	0.55 ab
Methionine 100	0.57 a	0.45 e
Methionine 200	0.46 ed	0.45 e
Aa 750	0.51 b-d	0.54 ab
Aa 1500	0.47 c-e	0.44 e
Control	0.45 e	0.34 f

AA (aminoacid containing fertilizer). Treatments that are shared with other letters are significant (P<0.01).

Table 3: Effect of source and amino acid concentration on acidity content.

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	73.030 a	35.576 e
Cysteine 200	62.424 bc	21.515 g
Methionine 100	63.636 bc	66.364 b
Methionine 200	59.091 cd	27.273 f
Aa 750	36.364 e	19.030 g
Aa 1500	21.81 fg	38.182 e
Control	65.455 b	55.152 d

AA (aminoacid containing fertilizer), Treatments that are shared with other letters are significant (P<0.01).

Table 4: Effect of source and amino acid concentration in Vitamin C (mg per 100 gr).

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	498 b-e	473.33 fg
Cysteine 200	436.67 hi	520 abc
Methionine 100	508.33 a-d	541.33 a
Methionine 200	454 gh	468.33 fh
Aa 750	508 a-d	482.67 defg
Aa 1500	489.33 c-f	525.33 ab
Control	413.33 i	460 fh

AA (aminoacid containing fertilizer), Treatments that are shared with other letters are significant (P<0.01).

Table 5: Effect of source and amino acid concentration on Yield (g per plant).

Mg.l	With the nitrogen 50 kg ha^{-1}	With the nitrogen 250 kg ha^{-1}
Cysteine 100	19.03 ab	17.76 de
Cysteine 200	19.03 ab	16.90 f
Methionine 100	18.65 bc	18.33 cd
Methionine 200	19.4 a	18.43 bcd
Aa 750	17.59 e	16.54 f
Aa 1500	18.19 ce	16.73 f
Control	19.61 a	18.33 cd

AA (aminoacid containing fertilizer), Treatments that are shared with other letters are significant (P<0.01).

Table 6: Effect of source and amino acid concentration on dry weight (%).

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